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## *Salmonella* Senftenberg Infections and Fennel Seed Tea, Serbia

**To the Editor:** The first documented outbreak of salmonellosis linked to consumption of plant products in the Autonomous Province of Vojvodina, Serbia, occurred from March 2007 through September 2008. Fourteen cases of *Salmonella enterica* serotype Senftenberg infection were reported.

The yearly incidence of salmonellosis in Vojvodina during 2003–2007 ranged from 25/100,000 inhabitants to 70/100,000 inhabitants; 34 outbreaks were reported in 2007, caused predominately by *S. enterica* serotype Enteritidis (*1*). Most outbreaks were associated with consumption of food of animal origin (*1*,*2*). *Salmonella* spp. were isolated from seeds in 2004, when *S. enterica* serotype Mbandaka and *S. enterica* serotype Virchow were isolated from sesame seeds (*3*).

Before 2007, *S.* Senftenberg had rarely been identified in Vojvodina. During 2003, 3 cases were reported. In 2004–2005, no *S.* Senftenberg cases were reported. In 2006, 8 cases of *S.* Senftenberg infection were reported among infants <12 months of age. An outbreak investigation did not reveal the source of infection. Common to all of those infected was their age and their consumption of infant formula. Nonetheless, laboratory analysis of samples of the various formulas did not show any pathogens. Two additional cases occurred in 2007 among patients who were <12 months of age. These cases confirmed suspicion that the infections had a source other than formula. Further investigation led to the consideration of tea consumption as a possible factor.

In April 2008, a total of 3 infants <12 months of age with salmonellosis came to the attention of investigators. *S.* Enteritidis was first identified in the samples of their feces. One month later, feces samples from the 3 infants were tested again, and *S.* Senftenberg was isolated from all 3 specimens.

After these findings, the Institute of Public Health of Vojvodina conducted an outbreak investigation in collaboration with institutes of public health at the district level. A case was defined as the presence of a laboratory-confirmed *S.* Senftenberg infection during 2007–2008. All case-patients (or their parents) were interviewed by using a standard questionnaire for salmonellosis, which was expanded to include questions regarding tea consumption.

A standardized method of enterobacterial repetitive intragenic consensus (ERIC)–PCR, based on the method of Versalovic et al. (*4*), with ERIC-PCR with ERIC2 primer (5′AAGTAA GTGACTCGGGTGAGCG-3′), was applied. DNA was isolated by using the InvitrogenPure Link Genomic DNA purification kit (Invitrogen, Carlsbad, CA, USA). Gene sequences were amplified in a Perkin/Elmer thermal cycler (model 9600) (PerkinElmer, Waltham, MA, USA). A DNA ladder was constructed by using Gene Ruler 100-bp DNA Ladder Plus (Fermentas, Glen Burnie, MD, USA).

Exploratory interviews with parents showed that all 3 infected infants had consumed commercially manufactured baby tea during the previous

month (after diagnosis of *S.* Enteritidis infection was made). Before feeding it to the infants, the parents had not heated the tea until it boiled, but rather had poured boiled water over the tea. After obtaining that information, we tested 33 samples of the incriminated brand of tea from public grocery stores and supermarkets; 13 samples were positive for *S.* Senftenberg. The organism's genetic profile was identical or similar from both tea and human samples (Figure).

Baby tea, widely distributed throughout Serbia, contains aniseed and caraway and fennel seeds. Sanitation inspectors collected samples from tea manufacturers. In the fennel seed sample, *S.* Senftenberg was identified. According to the tea manufacturer, fennel was purchased from another company, which collected seeds from individual producers. Fennel seed was cultivated in a household garden by an unregistered producer; neither the grower nor fennel stocks could be found. Two cases of *S.* Senftenberg from 2007 were retrospectively linked to infant tea, as were all other cases reported in 2008.

Demographic characteristics and clinical status of the case-patients were analyzed. Of 14 cases of *S.* Senftenberg infection, 10 were in infants <12 months of age (average 5.1 months). Half had diarrhea and the same proportion had fever >38.5˚C. Ten patients were female and 4 were male. All 4 adults had mild infection, except 1 adult who had concomitant *Clostridium difficile* infection. Three infants and an adult with concomitant infection were hospitalized.

Most infections were reported in May 2008, including the 3 cases in infants who were recovering from *S.* Enteritidis infection. After September 2008, no new cases of *S.* Senftenberg were reported until July 2009, when 1 case was identified in a 24-year-old man.

The heat resistance of *S.* Senftenberg is well known and is much higher than that for most other *Salmonella* serotypes (*5*). A number of recent outbreaks of *S.* Senftenberg infection resulted from consumption of fresh products. Thus, products that will be used in a fresh state should undergo more rigorous testing for pathogens, or better methods of infection control must be used.

The European Food Safety Authority has noted that all botanicals or botanical preparations could become hazardous as a result of flaws in the production process; therefore, manufacturers should follow the Hazard Analysis and Critical Control Point systematic approach (*6*). This system must be applied with the necessary flexibility and adapted to each botanical preparation on a case-by-case basis.

In 1999, the US Food and Drug Administration recommended that seeds be disinfected by washing with calcium hypochlorite solution before they sprout. However, this treatment destroys only pathogenic microorganisms on the seed surface (*7*,*8*). Thus, new methods, such as high hydrostatic pressure or use of bacteriophages as biocontrol agents should be adopted. High-pressure processing does not change the taste of food or cause any physical damage (*7*). With further refinement of phage delivery mechanisms, *Salmonella* phages could be effective in eliminating or reducing *Salmonella* contamination of vegetables (*9*).

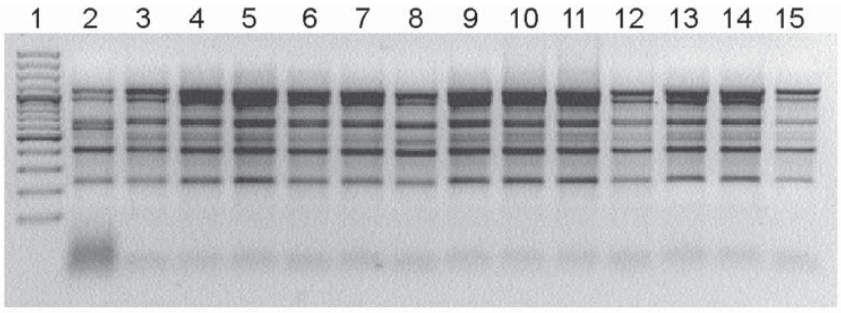


Figure. Enterobacterial repetitive intragenic consensus (ERIC)–PCR ERIC2 primers. Lane 1, molecular mass ladder; lanes 2–7, nonoutbreak isolates; lanes 8–9, isolates from baby tea; lane 10, isolate from fennel; lanes 11–15, isolates from salmonellosis patients. ERIC PCR with ERIC2 primer (5′-AAGTAAGTGACTCGGGTGAGCG-3′) was used. DNA was isolated by using the InvitrogenPure Link Genomic DNA purification kit (Invitrogen, Carlsbad, CA, USA). Gene sequences were amplified in a Perkin/Elmer thermal cycler (model 9600; PerkinElmer, Waltham, MA, USA). A DNA ladder was created by using Gene Ruler 100-bp DNA Ladder Plus (Fermentas, Glen Burnie, MD, USA).

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## Cryptosporidiosis Associated with Wildlife Center, Scotland

**To the Editor:** Handwashing is the single most important prevention step in reducing transmission of gastrointestinal zoonoses (*1*). Nevertheless, Health Protection Scotland receives reports of 500 to 700 laboratory-confirmed cases of cryptosporidiosis each year. Cryptosporidiosis symptoms include profuse, watery diarrhea, often accompanied by bloating, abdominal pain, and nausea. On April 15, 2005, National Health Service Tayside District's public health department called a meeting of the incident control team after a single index case of cryptosporidiosis in Scotland. One reported case rarely results in such measures; however, initial investigations determined that this case-patient may have acquired infection by contact with scouring (diarrhea) lambs at a wildlife center, during the Easter break (March 27–April 10, 2005). Subsequent public health actions included active surveillance of recent *Cryptosporidium* spp. laboratory reports, active case finding, the microbiologic analysis of feces/rectal swabs from lambs and bedding samples, and an assessment of the wildlife center's private water supply. Control measures included the removal of lambs from the center, disinfection of the premises with hypochlorite, and stopping direct contact between animals and visitors.

In total, 128 microbiologically confirmed cases were reported to the incident control team. An additional 252 clinical cases were reported among wildlife center visitors for whom no stool sample was taken. The illnesses of these persons had a similar implied incubation period (typically 6–7 days) and their age profiles were the same as patients with laboratory-confirmed cases. Of 128 patients with confirmed cases, 117 visited the wildlife center, and infections of the remainder were attributed to secondary spread. Most case-patients were Tayside residents and were generally resident in towns and villages near the wildlife center. Of the 128 human isolates, 103 were identified as *Cryptosporidium parvum*. Oocysts from the environmental samples (lamb pen drain and central drain debris) were also identified as *C. parvum*. Isolates could not be obtained from lambs because the lambs had died and were subsequently incinerated by the wildlife center. Although assessment of the private water supply revealed unacceptable levels of coliforms, oocysts were not detected.

Daily gate receipts for the wildlife center were obtained. Using these as a denominator for confirmed cases, we calculated the daily attack rate. The attack rate peaked at 8.1% on April 8, 2005. The relative risk for visiting the wildlife center over the defined period was estimated to be ≈13.3 for confirmed *Cryptosporidium* infection. In view of the strength and clarity of the association between visiting the wildlife center (petting lambs in particular) and being a case-patient (Figure), no formal analytical epidemiologic investigation was conducted.

These results suggest that the outbreak was caused by direct contact with scouring lambs, a recognized risk factor for cryptosporidiosis, coupled with inadequate handwashing facilities (*2*,*3*). Anecdotal reports indicate that children were encouraged to pick up lambs from the farm enclosure, despite visible signs of diarrhea on the animals. The lack of handwashing facilities in this wildlife park was surprising because the Scottish government had conducted an information campaign that Spring (March), encouraging primary prevention initiatives, specifically in petting farms and zoos, and recommending the provision and use of handwashing facilities (www.infoscotland.com/handsclean/CCC_FirstPage.jsp). Moreover, no hand-